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AUTHOR(S):

YASUHIRA, Kimio; ASADA, Takaaki; NAGANO, Kotoko

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SOME PROBLEMS ON PASSIVE TRANSFER OF TUBERCULIN SENSITIVITY

Kimio YASUHIRA, Takaaki ASADA and Kotoko NAGANO
安平公夫　浅田高明　永野琴子

The Second Division of the Tuberculosis Research Institute, Kyoto University

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I. Introduction

Landsteiner and Chase (1942) who were studying the sensitization of animals with simple chemical compounds found that some of the drugs had the ability to produce a delayed reaction in the skin and the reaction was transferable by cell transplantation from sensitized to nonsensitized animals. After that, Chase (1945) reported that the tuberculin sensitivity was also transferable by peritoneal exudate cells from sensitized animals to recipients. The cells were obtained from guinea pigs sensitized with dead tubercle bacilli in oil emulsion five to nine weeks before the treatment. The reactions in recipients were positive in 16 out of 17 cases in his report. This report called attention in the field of immunology and many studies were carried out in order to confirm the success, because it had generally been considered that the tuberculin reaction had been different from ordinary allergic reactions in its inability to be transferred into recipient animals by transfusion of sera from sensitized animals. Only two years after the enlightening report, Kirchheimer et al (1947) raised an objection to the report and published that the successful transfer of tuberculin sensitivity was mediated with cells from animals sensitized only with living bacilli. Afterwards many reports have been published the possibility of the cellular transfer of the tuberculin sensitivity with cells from animals sensitized with dead or living bacilli.

There may be two weak points common to these experiments. The reactions in recipients were usually rather weak compared with those of actively sensitized animals. They were about 10 mm in diameter in general and about 15 mm of maximum. In addition, guinea pigs were used as the recipient animals in these experiments. Guinea pigs may not be suitable to test skin reactions in cell transplantation experiments, because their skin is so sensitive to the Shwartzman-type of reaction which is probably assocciated wiht the transplantation experiments and also edema is often stronger than erythema.

The present authers' experiments was carried out to elucidate the ability of sensitized cells in the transfer of the sensitivity. The results obtained were very complicated and some of them may require further studies. But some important problems were made clear with regard to the cellular transfer of tuberculin sensitivity.

II. Local passive transfer of tuberculin sensitivity

Sensitized cells were prepared from peritoneal exudates of adult rabbits, guinea pigs, and rats sensitized with dead tubercle bacilli in oil emulsion. The cells were mixed with the same amount of 1:5 diluted old tuberculin (OT). 0.2 ml of the mixture was injected into the skin of nonsensitized recipient animals. The cutaneous reactions were observed successively at definite intervals after the treatment. Details of the methods and some results of the experiments have already been published by one of the present authors (1959), so the results are shortly summarized as follows:

i) When the sensitized cells were introduced with OT into homologous animals, the reactions of the skin usually appeared about 3 hours and reached the maximum about 6 hours after the treatment. The reactions diminished after 48 hours leaving slight colorings at the sites of the treatment. The reactions were as large as 15 mm in diameter at the highest and stronger than 7 to 8 mm control reactions.

ii) When the cells were injected into the skin of heterologous animals, the reactions appeared more intense and more delayed in comparison with those of homologous animals. The maximum point of the reactions was observed about 48 hours after the treatment and sometimes the reactions were over 20 mm in diameter with apparent redness and swelling.

iii) The cells reduced gradually their ability to produce the reaction when the administration of the cells was retarded after the preparation of the cells from donors. For instance, the incubation of the cells with OT at 37°C for 5 to 10 hours made the reactive ability of the cells diminish.

iv) After centrifugation of the mixtures of the sensitized cells and OT, the supernatant had no ability to induce the reaction, although the precipitated cells remained the same ability as the original mixtures.

v) Beside the above-mentioned experiments, cells from the spleen, lungs and liver were injected into recipients instead of the cells from peritoneal exudates. It was also pointed out that some of these cells had the ability to produce a very slight reaction.

These experiments were similar to those of Metaxas and Metaxas-Bühler

(1948, 1955) following Prausnitz-Küstner's skin test for antibody detection with some modifications. The method was, however, defective because of provocating a intensive local irritation by the transfered cells, though the irritation sometimes increased the reaction and this contributed to the detection of a small amount of antibodies. So it was neceessary to do the experiments of ordinary method of systemic sensitization transfering the sensitized cells into vessels or the peritoneal cavity of recipient animals.

III. Intraperitoneal transfer of tuberculin sensitivity with cells from animals sensitized with dead bacilli.

Rabbits were used in this experiment taking sensitized cells from thoracic lymph as well as from lymph nodes, spleen and peritoneal exudates.

Sensitization of animals was carried out as follows; adult rabbits were treated twice with subcutaneous injections of 5 mg of heat-killed tubercle bacilli (H37Rv strain) in oil emulsion. Rabbits with strong tuberculin reactions were used as donors about 3 weeks after the last treatment for the seusitization. Peritoneal cells of donors were obtained irrigating with Tyrode solution the peritoneal cavity 4 or 5 days after the intraperitoneal injection of about 100 ml of liquid paraffin. The cells were consisted of about 80 per cent of mononuclear cells and 20 per cent of lymphocytes and polymorphonuclear leucocytes. About 0.5 ml of packed cells were obtained from each donor. Cells from three donors were injected into the peritoneal cavity of a recipient. Skin tests were performed 36 hours after the cell transfer and negative reactions were observed in all of the recipients. Lately we noticed that the negative data had resulted from overlooking faint and evanescent reactions in recipients, because we expected the apparent reactions as would have been observed in actively sensitized donors. The reactions in recipients were recognized as very slight redness of about 10 mm in diameter at the highest. Sometimes they were no more than the local dilatation of vessels diminishing when the reactions were examined.

After these preliminary experiments, sensitized cells from the spleen, lymph nodes, and thoracic lymph were separately transferred intraperitoneally into recipient rabbits. Six donors were fixed on the boards on their back at the same time. Each thoracic lymph duct was exposed at the entrance of the duct to the left jugular vein. Thoracic lymph was collected in a chilled beaker with a small amount of heparin solution. About 30 to 40 ml of lymph were obtained from a rabbit in 3 to 4 hours. About 10,000,000 cells were usually contained in one ml of the lymph. The cells were consisted of about 98 per cent of lymphocytes and a small amount of monocytes or polymorphonuclear leucocytes. Almost all of these cells

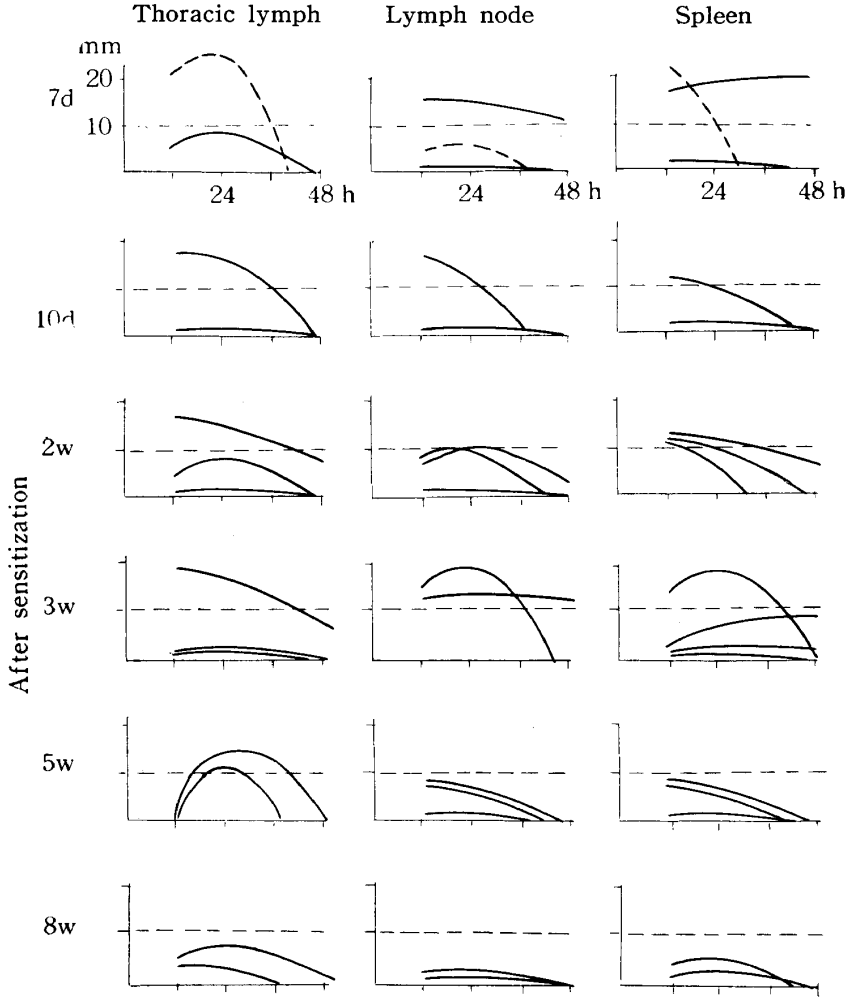
were alive at the time of the transplantation. After collecting the thoracic lymph, all rabbits were killed by bleeding from the carotid artery to remove spleens and peritoneal lymph nodes. After being trimmed off fat, the cells were teased out from fine pieces of the organs in chilled and heparinized Tyrode solution with about 10 per cent of normal rabbit serum. After filtrating through 40 layers of a brassy net, the cell suspension was used for the transplantation as described by Harris et al (1954). The resulting suspension contained mainly of single cells, and appeared in supravital staining with neutral red and Janus green to comprise more than 85 per cent of lymphocytes and some of monocytes, histiocytes, fibrocytes and others. These cell suspensions obtained from 6 to 9 rabbits were divided into two parts and transplanted into two white rabbits. Those cells were obtained from donors 7 and 10 days and 2, 3, 4 and 6 weeks after the treatment for sensitization, with the consideration of the ability of sensitized cells to be changeable with times after the treatment. The skin tests in recipients after cell transplantation were no more than negative with a few exceptions. Some of the reactions seemed to be slight positive with a character of evanescent reaction diminishing untill 36 hours after the test injection and sometimes remaining yellowish colored at the site of the test. Therefare, we concluded that the reactions were not apparent enough in recipient animals to be a sign of transferred tuberculin sensitivity, although the reactions were completely negative when the cells from nonsensitized animals were transferred into recipients.

IV. Intraperitoneal transfer of tuberculin sensitivity with cells from animals sensitized with living bacilli

Experiments were carried out in the same manner as those described above with the exception of the sensitizing method of donors. Adult rabbits were sensitized with 2 mg of living tubercle bacilli (H37Rv strain) in saline suspension. A half of the suspension was administered into ear vein and the other into the subcutaneous tissues of foot pads. Tuberculin sensitivities in half of these animals were positive on the fourth day to one week and developed quite extensively 2 weeks after the infection.

Transferred sensitivity seemed to be more apparent in these experiments than those immunized with dead bacilli. About one-third of the animals were positive though they were still weak. The reactions reached at their maximum 12 to 24 hours later. Therefore, it was made clear that lymphocytes from lymph nodes and thoracic ducts and the cells from spleens had participated in the transfer of the sensitivity. The sensitizing ability of donor cells decreased with the lapse of time between sensitization of animals and the removal of the cells as shown in Fig. 1.

Fig. 1. Tuberculin Reactions after the Passive Transfer of Cells from Sensitized Rabbits with Living Tubercle Bacilli.



Cells from bone marrow and appendices had no ability of transfering the reaction from donor to recipient.

V. Passive transfer of tuberculin sensitivity with sensitized lung cells.

It is worthy of mention that histological changes were observed in donors when the sensitized cells were obtained. The changes were of course of tuberculous nature with or without tubercle formation. We could find easily some epithelioid cells, giant cells or monocytes with epithelioid character among the cells from donor animals. Therefore, it was thought necessary to confirm the ability of epithelioid cells of transferring the sensitivity.

The sensitized lung cells were obtained by the method described by Myrvik et al (1961). Remarkable tuberculous lesions were produced in the lungs of sensitized rabbits by the challenge injection of about 3 mg of tubercle bacilli in a saline suspension into an ear vein of the animals.

The lesions seemed to be granulomatous and consisted of numerous large exudate cells of epithelioid character packed in alveoli and small branches of bronchi. On the fourth or fifth day after the challenge injection, the lungs with the bronchus were removed from the corpse after irrigation with a large amount of Ringer's solution from the right chamber of the heart.

Lung cells were obtained by repeated washings of the resected lungs with a large quantity of Hank's solution poured into the bronchus. About 2 or 3 ml of packed cells were obtained from the lungs of a sensitized rabbit. The cells were introduced into the peritoneal cavity of the recipient after calculation of the cell amounts. About 75 per cent of the cells were mononuclear cells of epithelioid character and showed a fine rosette stainable with neutral red in their cytoplasma. Others consisted largely of lymphocytes and rarely giant cells and polymorphonuclear cells.

Results of the test in recipients are shown in Table 1. Positive transfer of the sensitivity was seen in about one-third of the tests. Transferred sensitivity by the lung eclls was not so strong as those by the other cells.

Table 1. Intraperitoneal Transfer of Tuberculin Sensitivity by Lung Cells.

Donors			Tuberculin reaction of recipient			
sensitization	challenge	sacrificed after the challenge	12 hours	24 hours	36 hours	48 hours
			mm			
dead H37Rv+Adj	living BCG	6 days	(16×4)	0	0	0
〃	〃	〃	(2×3)	0	0	0
〃	〃	4 days	0	0	(10×10)	0
〃	dead H37Rv	〃	(5×5)	(3×3)	(3×2)	0
living H37Rv	〃	〃	7×7	15×10	10×6	(9×10)
〃	〃	〃	0	0	0	0
〃	dead BCG	2 days	(6×6)	0	0	0
〃	〃	4 days	10×10	5×5	8×6	†
dead BCG+Adj	〃	〃	20×15	15×10	8×8	9×9

Note: brackets mean faint reactions.

VI. Discordance of transferable sensitivity from the tuberculin reaction of donors

As seen in Table 2, it is sometimes observed that a positive reaction appeared in recipients which had been received with cells from tuberculin negative donors early after the sensitization and the conversed phenomena by the cells from tuberculin positive donors. This may suggest that the tuberculin reaction of animals has no exact correlation with their sensitized situation. One of the most apparent examples of the discrepancy was in the experiments of Asada (1959):

the rats did not manifest tuberculin reaction even after a vigorous treatment by tubercle bacilli but the cells had the ability of transferring the sensitivity into rabbits or guinea pigs.

VII. Different reactivity of the skin of recipients to the tuberculin reaction

It has also been shown in Table 2 that the tuberculin reaction appeared in one recipient and didn't in the other when the cell suspension was divided into

Table 2. Some Cases of Cellular Transfer of Tuberculin Sensitivity in Early Stages of Sensitization of Donors.

Donor No.	Sensitization	Days After	Tuberculin Reac.	Precipit. Titer	Hemaggl. Titer	Transfered Cell	Recipient No.	12 hours	24 hours	36 hours	48 hours	Precipit. Titer	Hemaggl. Titer
2	living H37Rv	7	+	4	64	Spleen	101	20×13	17×14	(20×17)	(23×18)	0	0
3	〃	〃	±	0	16	〃	102	(25×13)	(18×8)	0	0	0	(2)
4	〃	〃	—	0	64	Lymph Node	103	0	(5×5)	0	0	0	0
5	〃	〃	—	0	32	〃	104	20×15	15×15	(15×14)	(12×11)	0	0
6	〃	〃	+	0	16	Thoracic Lymph	105	(6×5)	(9×8)	0	0	0	0
7	〃	〃	+	0	32	〃	106	(21×21)	(29×25)	0	0	0	0
1	〃	10	—	0	32	Spleen	113	13×10	13×9	0	0	0	0
9	〃	〃	—	0	4	Lymph Node	111	(17×15)	(13×14)	0	0	0	0
8	〃	〃	—	0	64	Thoracic Lymph	109	19×13	14×11	0	0	0	0
10	〃	〃	+	0	64	Spleen	114	0	0	0	0	0	0
11	〃	〃	+	0	64	Lymph Node	112	0	0	0	0	0	0
12	〃	〃	+	2	64	Thoracic Lymph	110	0	0	0	0	0	0
13	〃	14	—	0	16	Spleen	117	14×12	13×11	(10×9)	(6×4)	0	0
14	〃	〃	—	0	32	Lymph Node	118	(10×8)	(12×8)	(8×6)	0	0	0
15	〃	〃	±	(2)	64	Thoracic Lymph	121	22×16	10×11	12×10	(9×8)	0	0
16	〃	〃	++	(2)	64	Spleen	120	(15×12)	(7×7)	(5×5)	0	0	0
17	〃	〃	++	(2)	64	Lymph Node	119	(7×7)	(9×10)	(9×7)	(3×3)	0	0
18	〃	〃	++	0	32	Thoracic Lymph	122	(4×4)	(8×7)	(4×4)	0	0	0

two parts of the same amount and was transferred into two recipients. This shows the different skin reactivity of recipient animals to the tuberculin test or the different internal status of recipients to the development of transferred sensitivity. It is true that the positive tuberculin reactions were observed usually only in a part of the recipients in many experiments.

VIII. Tubercle bacilli in the transfering cell suspensions

As a rule in the experiments mentioned above, the test injections of OT were usually carried out in recipients 36 hours after the cell transfer and the reaction observed were faint and evanescent even if positive. When the tuberclin reaction was repeatedly performed in the same animals, the reactions became more apparent with time after the cell transfer as shown in Table 3. The reactions after some weeks were similar in their nature and intensity to those of

Table 3. Tuberculin Reaction after the Transfer of Cells from Animals Sensitized with Living Bacilli.

After the transfer (weeks)	1	2	3	4	5	6	7	9	12
Thoracic Lymph	÷ —	++ —		++	+		++	++	
Lymph Nodes	++ ++	+ +++		++		—			
Spleen	++ +	+ ++		+ ++ +++		++			
Bone Marrow	÷	+++	+	++	—		++		
Various Cells (into the Skin)	+— —— ——	++— ÷ +++	+± +++ —	+++ +++ ++++	+++ ++ +		++		+

Table 4. Tubercle Bacilli Cultured from the Transfering-materials of the Animals Sensitized with Living Bacilli.

After the infection	7d	8d	10d	2w	3w	5w	8w
Thoracic Lymph (0.002 cc of cell amount)	—	—	—	—	—	—	—
Lymph Nodes (0.02 cc of cell amount)	+	++	++	+-	—+	+	—
Spleen (0.02 cc of cell amount)	—	+	+	+—	—+	—	—

÷ colony count 0–10
+ » 11–50
++ » 50<

actively sensitized animals. These positive reactions led to the possibility of contamination of antigens in the transferred meterials. In truth, Table 4 shows the colonies of the tubercle bacilli in the materials isolated on Ogawa's egg-media. Negative cultures of thoracic lymph may be due to too small amounts of cultured materials, since the animals transferred with thoracic lymph showed as strong reactions after a period of over two weeks as those of the actively sensitized animals.

In animals injected with the sensitized cells immunized with dead tubercle bacilli, the positive reactions were occasionally observed only when their sensitivity was tested a few weeks after the cell transfer. Therefore, it should not be denied the possibility of active sensititization with the antigens (dead bacilli) introduced into the recipients.

Now it is necessary to distinguish the active from passive sensitization in cellular transfer of tuberculin sensitivity. Positive reactions were easily recognized in many animals 4 days after sensitization with dead bacilli with adjuvant or living tubercle bacilli. The positive reactions were also demonstrated in a half of the sensitized animals even 2 days after the sensitization, if the animals were sensitized with a smaller amount of bacilli as shown in Table 5. The reactions

Table 5. Tuberculin Reaction and Serum Antibodies (In the Early Stages of Infection)

Rabbit No.	Sensitization	2 Days after the Sensitization			4 Days after the Sensitization		
		Precipitation	Hemagglut.	Tuberculin React.	Precipitation	Hemagglut.	Tuberculin React.
502	Dead H37Rv 10 mg	0	0	(4×4)	0	4	(5×2)
503	〃	0	0	(5×5)	0	8	(6×4)
504	〃	0	2	(3×3)	2?	2	0
505	〃	—	2	(4×4)	0	2	0
506	〃	0	0	0	—	8	(9×7)
507	Dead H37Rv 0.1 mg	0	2	(4×4)	0	8	(5×5)
508	〃	0	2	12×17	4	128	(17×15)
509	〃	0	0	(10×10)	2?	2	16×12
510	〃	0	0	(10×10)	0	0	(14×14)
511	〃	0	2	(8×8)	0	4	0

of these animals in the early stages of active sensitization were similar to those of the recipients with passively sensitized reactivity in their evanescent character. So it is impossible, at present from these results alone to decide which was more essential for the positive reaction of the recipients, active or passive immunization.

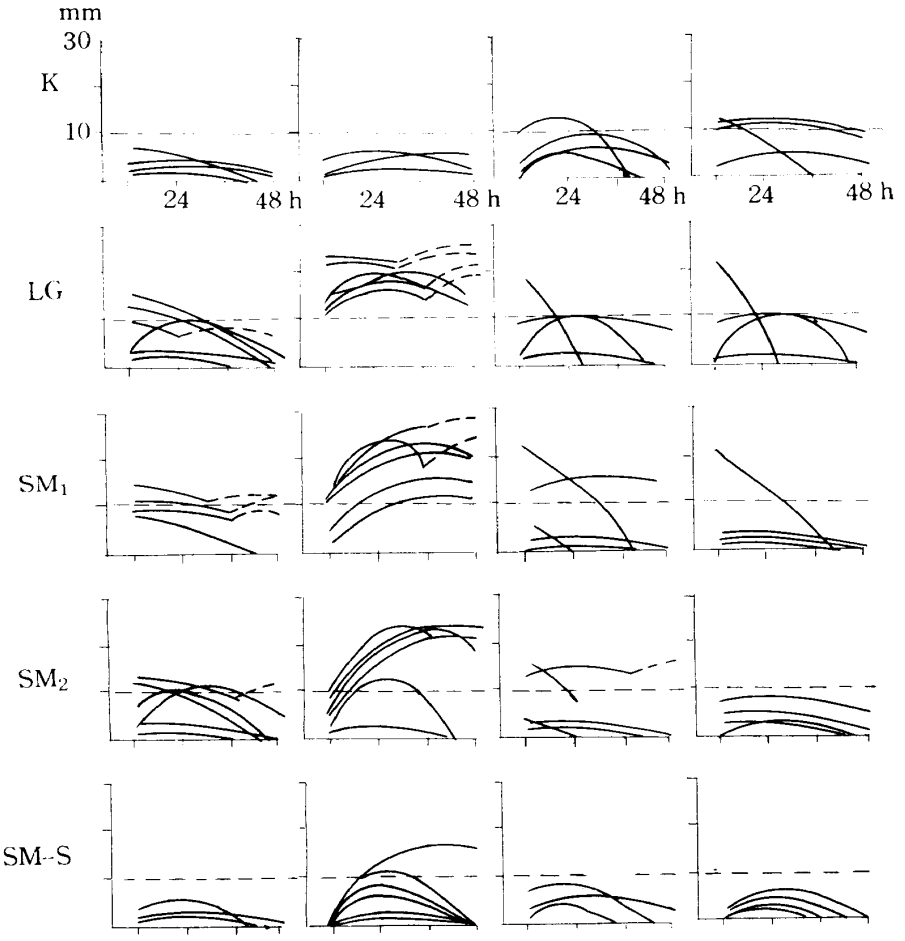
IX. Ability of cell fragments to transfer tuberculin sensitivity

Possibility of passive transfer of the tuberculin reaction by fragments of sensitized cells was reported from our laboratory (1958). In this experiment, peritoneal exudate cells in a 0.25 M solution of saccharose were disrupted by a glass homogenizer. Cell fragments were prepared by the differential centrifugation in accordance with the ordinary schedule. The fragments were tested as to their ability to transfer the tuberculin sensitivity by the local transplantation method described above. Positive reactions appeared at the site of the skin at which mitochondrial and microsome fractions were injected with diluted OT. Nuclear

and supernatant fractions of the sensitized cells were negative in their ability to reveal reactions.

After that, fragments of lymphocytes from lymph nodes and thoracic lymph of sensitized rabbits were tested by the same method. In this experiment, positive reactions also resulted by the mitochondria and microsomes of the cells (Fig. 2). Microsomes were separated into two parts; the large particles with about 100 S

Fig. 2. Tuberculin Reactions of Rabbits Passively Transferred with Cell Fractions.



Note: K, nuclei, LG, mitochondria, SM_1, microsome (100S), SM_2, microsome (40S), SM-S, supernatant

of the sedimentation rate and the small particles with about 40S. Both particles had the similar ability of provocating the reaction. A protein fraction was isolated from the supernatants by adjusting the pH of the solution at 5.2. This soluble protein was called as "pH 5.2 enzyme". This enzyme had no stimulating effect for the provocative ability of the skin reaction of mitochondrial and microsome fractions.

There may be two problems remaining in these experiments. One is the contaminating antigens (tubercle bacilli) in the transferred cell fractions. Intensive reactions as those of actively sensitized animals were easily recognizable in the recipients injected with these fractions some weeks after the treatment. This strongly suggests that some living tubercle bacilli or their antigenic fragments were contained in some of the cell fractions. The other is the presence of some chemical factors in the fragments which stimulate the reaction. It is well known that a large amount of histamine is contained in particles in the cells. It may be necessary in these experiments to avoid the stimulating effect of histamine or histamine-like substances contained in the cell fragments. Sometimes positive skin reactions could be observed even in animals injected with cell fragments from nonsensitized animals, though the reactions were less intense than those of sensitized cells.

X. Summary

Many experiments have been carried out on the cellular transfer of the tuberculin sensitivity. The results of the present authors' exprements may call additional attention to (1) the antigens for the sensitization of donors: living tubercle bacilli were more useful for the antigen than dead bacilli, (2) the interval after the sensitizing treatment: the sensitizing ability of donor cells decreases with the lapse of time between sensitization of the animals and the removal of the cells. (3) Skin reactivity of animals: in donors, the skin reactivity does not always go in parallel with their state of hypersensitivity. In recipients, the skin reaction appears sometimes differently to each other, even if they were given with the same material. (4) Contamination of antigens in transferred materials: some of the reactions considered due to the passively transferred sensitivity may be caused by the active sensitization of animals indisputably, and (5) the ability of cell fragments to produce the sensitinity: mitochondria and microsomes of sensitized cells as well as whole cells have the ability to produce skin reactions in recipients.

Recently the role of lymphocytes has come into notice in their ability to transfer tumor immunity or homotransplantation immunity from immunized to normal animals as reported by Mitchison (1953) and Billingham (1954). Do lymphocytes take part in the cellular transfer of the tuberculin sensitivity as well as in these immunities, as they are quite similar to each other in the absence of antibodies in sera of sensitized animals? Fukase et al (1953) have already reported the possibility of cellular transfer of the tuberculin sensitivity by lymphocytes from lymph nodes or thoracic lymph of sensitized animals. We could confirm

this result in the present experiments, but also recognized that the ability of the lymphocyte was not superior to the other cells, for instance, to splenic cells, peritoneal exudate cells or lung cells of sensitized animals. It may be unreasonable to restrict the ability of the cellular transfer of tuberulcin sensitivity to one type of cells.

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